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Yeast: bridging the gap between phenotypic and biochemical assays for high-throughput screening

Abstract:

Introduction:

Both *in vitro* biochemical and phenotypic assay platforms have clear limitations in high throughput screening (HTS) for drug discovery. The use of genetically tractable model yeast as a vehicle for target-based HTS overcomes many of these by allowing the identification of on-target compounds that function within a eukaryotic cellular context.

Areas covered:

In this Special Report, the use of yeast-based assays in HTS is discussed with reference to the various platforms that have been utilized over the past 20 years. The specific issues considered are the necessity to employ counter and secondary screening approaches to ensure the on-target activity of hits, and the recent developments in detection systems that have facilitated miniaturization and ultra-HTS.

Expert opinion:

The demonstrable possibilities of optimizing yeast-based HTS, coupled with the demonstration of utility in an industrial setting, shows that these platforms have the potential to *bridge the gap between phenotypic and biochemical assays for HTS*.

Keywords:

Yeast; Eukaryotic; High Throughput Screening; Drug Discovery

1. Introduction:

High throughput screening (HTS) facilitates the analyses of vast numbers of compounds ($\geq 100,000/\text{day}$) for biological activity, an approach central to modern drug discovery efforts. HTS assays formats can, in general, be divided into two categories: *in vitro* biochemical and cell-based, phenotypic assays. The former are often simpler and more specific, whereas cell-based phenotypic assays can be more challenging but provide cellular context [1].

For the automated HTS required to achieve true high content screening, all assays must be as simple as possible. For example, *in vitro* biochemical assays must produce a readily detectable and quantifiable signal (fluorescence, radiochemical etc) without complex substrate-product separation. This necessitates the isolation of pure, soluble and enzymatically active protein, clearly these requirements can be major obstacles [2, 3]. In contrast, cell-based phenotypic HTS platforms do not require target protein purification nor any knowledge of the target or targets, and compound activity is established in a cellular context that mirrors the *in vivo* scenario. For example, the necessity for a compound to cross membrane barriers to reach an intracellular target, thus predicting those compounds least likely to exhibit problems with stability or solubility *in vivo* and fast-tracking drug-like entities for further development. However, these advantages can also be detrimental. For example, compounds that fail to cross membrane barriers and reach the target could represent promising early stage hits, and their loss precludes further development. In addition, the non-target-based phenotypic readout requires that target deconvolution is approached *a posteriori* to facilitate rational development of compound hits [2, 3].

In summary, both approaches have distinct challenges which are summarized in Table 1. However, to best reflect the physiological context in which a compound has an effect, cell-based assays have obvious advantages [4]. Engineering the cell to provide a target specific readout is possible, thereby combining the advantages of both *in vitro* biochemical and cell-based, phenotypic assays. However, target eukaryotic cells can be difficult and expensive to culture and genetically manipulate [5], in addition the process redundancy found in complex systems can confound the readout of HTS for a specific inhibitor [6]. Therefore, whilst cell-based systems clearly provide some significant advantages compared with conventional target-based *in vitro* approaches, their use is not without limitations.

Yeast, particularly *Saccharomyces cerevisiae*, but also *Schizosaccharomyces pombe*, have been long established as relatively simple, tractable and highly characterised model eukaryotes [7]. Yeast benefit from a highly developed genetic tool kit and are fast growing (doubling time ~ 90 minutes) in simple, low-cost (liquid or solid) media. Such characteristics make *S. cerevisiae* and *S. pombe* ideal for antifungal HTS [8]. However given that many

cellular pathways are conserved in yeast and other eukaryotes (e.g. humans) these model cells can be easily engineered for the analyses of orthologous functions [9]. For example, the use of the yeast two hybrid systems has been utilized to identify inhibitors of protein-protein interactions [10, 11]. However, the application of this technology for drug discovery has been relatively limited. In the following sections I will focus on the wider use of yeast-based platforms designed to identify inhibitors of protein function. The predominant platforms have been previously described and illustrated [2, 3], and in the following sections the contribution of yeast to HTS and drug discovery will be further discussed with reference to the available literature (Table 2, in chronological order) and the future perspectives considered.

2. Yeast HTS platforms

2.1 Lethal expression

Given the highly developed genetic tool kit available to modify the yeast genome and express axenic proteins, both *S. cerevisiae* and *S. pombe* have been successfully engineered into reporter systems that have been utilized in HTS. For example, expression of the influenza virus ion-channel forming protein in *S. cerevisiae* is lethal [12]. The expression of pro-apoptotic HIV-1 protein Vpr is similarly lethal to the fission yeast *S. pombe* [13]. For both cases, this lethal phenotype has been utilized to identify molecules that rescue the yeast, i.e. inhibit orthologous protein function. Lethal expression has also been used in yeast-based screens for modulators of human proteins [4, 6]. For example, the human kinase p38 α [14] and phosphatidylinositol 3-kinase (PI3K) [15] are both associated with oncogenesis (and a plethora of other diseases, Table 2) and their expression is toxic to *S. cerevisiae* [16, 17], as is the expression of α -synuclein (α -syn) associated with Parkinson's disease [18, 19, 20]. Similarly, recent work described an *S. cerevisiae* lethal expression platform in which yeast deficient in uridine transport were made susceptible to toxic 5-FUrd by the expression of Nucleoside Transporter 1 from the malaria parasite *Plasmodium falciparum*. These were then utilized in HTS to identify antimalarial compounds [21]. Importantly, in all phenotypic rescue assays the possibility of generically toxic false positives is removed greatly simplifying the generation and interpretation of data.

2.2 Transactivation

Lethal expression-based platform readout is reliant on positive growth changes in response to the direct chemical modulation of the target protein. However, given the extreme genetic tractability of both *S. cerevisiae* and *S. pombe* it is possible to engineer these model yeast to provide an indirect HTS readout through transactivation. Several examples have been reported [22, 23] (Table 2), but the most extensively described and HTS utilized example of a

yeast transactivation platform has been directed towards the discovery of mammalian phosphodiesterase (PDE) inhibitors [24, 25, 26, 27, 28]. The approximately 100, tissue specific, mammalian PDE isoforms all catalyze the conversion of cAMP and cGMP to the cyclic secondary messengers 5'AMP and 5'GMP. Given the tissue specificity of many of these isoforms, selective modulation has been recognized as having therapeutic potential for a wide range of human diseases [29, 30] (Table 2). *S. pombe* can tolerate deletion of adenylate cyclase (*cyr1*) and PDE (*csg2*). In their absence, exogenous cAMP or cGMP activates Protein Kinase A (PKA) and *ura4* expression from an introduced selectable marker is repressed. In the absence of *ura4* expression, the yeast can grow in the presence of 5-fluoroorotic acid (5FOA), which is converted to toxic fluoroorotidine monophosphate in the presence of URA4 [28, 31]. However, expression of yeast (*Cyr1*) or a mammalian PDE facilitated the conversion of cAMP or cGMP to 5'AMP or 5'GMP, leading to *ura4* expression and 5FOA sensitivity. Inhibition of PDE function rescued growth and the system has been formatted into a positive selection HTS used to screen libraries >200k in size [24, 25, 26, 27, 28]. To ensure specificity against single or small numbers of PDE isoforms screening against yeast expressing different isoforms can be undertaken, so called counter screening. Using this approach, many of the hits were demonstrated to be specific for one or more PDE isoforms [24, 25, 26, 27, 28]. Again, like the lethal expression platforms, these phenotypic rescue assays reduce the likelihood of false positive hits.

2.3 Substitution

Despite the advantages (e.g. reducing false positives) of lethal expression and transactivation platforms, the majority of reported yeast-based HTS have relied on the simple substitution of an essential yeast function with the orthologue from the organism of interest. Of course, this is dependent of the conservation of protein function between the yeast and an often distantly related eukaryote. This approach has been widely used in HTS of mammalian targets [9]. For example, mutant *S. cerevisiae* complemented by expression of a human potassium channel protein (Kir2.1) was formatted into an HTS assay where inhibition at low but not high potassium was scored as a hit [32]. Similarly, with a view to discovering hit compounds towards the treatment of obesity, human acetyl-CoA carboxylase 2 (AAC2) inhibitors were identified by HTS using mutant *S. cerevisiae* reliant on expression of human AAC2 [33]. More recently, a *S. cerevisiae* substitution platform was employed for HTS to identify inhibitors of human adenine nucleotide translocase 4 (ANT4), a target for cancer and cardiovascular disease amongst others [34]. In both cases, counter screening against yeast complemented with isoforms of the target (human AAC1 and ANT1, -2 and 3 respectively) ensured selectivity [33, 34].

However, the first report of the use of a substitution platform in HTS was against the ornithine decarboxylase (ODC), a key regulatory enzyme in eukaryotic polyamine (PA) biosynthesis, from *Haemonchus contortus*, a nematode worm that is a major parasite of ruminants. Similar to above, the platform utilized a *S. cerevisiae* ODC deficient mutant complemented by expression of the *H. contortus* orthologue [35]. Subsequently, recent years have seen most published activity using *S. cerevisiae* substitution platforms directed to enable HTS against parasitic proteins [2, 3]. For example, *S. cerevisiae* mutant yeast have been complemented by the expression of dihydrofolate reductase (DHFR), *N*-myristoyltransferase (NMT), phosphoglycerate kinase (PGK) and several other targets (Table 2) from protozoan and helminth parasites [36, 37, 38]. An *S. cerevisiae* substitution platform has also been used in HTS to identify inhibitors of the anti-leishmanial drug target inositol phosphorylceramide (IPC) synthase [39, 40, 41]. In this case, in the absence of a mammalian orthologue [42, 43, 44, 45], counter screening was performed against the mutant complemented with the yeast orthologue AUR1 [41] to remove off-target, generically cytotoxic compounds.

3. Detection and sensitivity

Many of the initial studies utilized agar plate-based assays to create reporter systems [46, 47, 48, 49]. However, it is clear that the measurement of growth on agar plates can only be semi-quantitative, even when colourimetric [46, 47]. Therefore, the majority of yeast-based HTS now establish growth in liquid culture in microtiter plates, via optical density, colourimetry, fluorimetry, or a combination of these [21]. The great advantage is that this liquid format is amenable to miniaturization from 96-well to 384-well, and even to 1536-well [41] plates (Table 2). The vast majority of assays have used turbidity (measured via optical density) as a measure of liquid growth [2, 3], sometimes with measures to enhance sensitivity such as deletion of efflux pumps, to reduce expulsion of potential inhibitory compounds, and non-lethal disruption of the plasma membrane [16]. However, a fluorescent readout provides considerably greater sensitivity [50] and has been recently employed in yeast-based HTS. For example, Bilisland *et al* (2013) employed fluorescent tags to facilitate multiplexing, thus allowing compounds to be screened against protozoan DHFR, NMT or PGK (Table 2) simultaneously with the human orthologue in a single well of a 384-well plate [37]. More recently, fluorescein di- β -D-glucopyranoside (FDGlu; a highly sensitive substrate for the glucosidases found in the yeast cell wall) was utilized to measure yeast growth in a HTS campaign against the *Leishmania* IPC synthase. The sensitivity of this approach allowed the assay to be miniaturized into 1536-well plates for screening against 1.8M compounds [41], to date the only published example of a yeast-based ultra-HTS.

4. Secondary assays

A significant advantage of the transactivation and lethal expression platforms described above is that they rely upon positive selection, thus reducing the number of generically toxic false-positive hits. In contrast, the negative selection employed in substitution assays necessitates counter screening in eliminate false-positives. However, despite this clear advantage, all yeast-based platforms have the inherent possibility of producing false-positive hits, for example due to inhibition of other elements within a biosynthetic pathway [2, 3]. Therefore, as with all HTS, secondary screens to ensure that the identified hits are *on target* are an essential tool is the discovery process. These can be *in vitro* enzymatic and/or cell-based assays. For example, some hits identified in the multiple HTS (3k to >200k compounds) using PDE transactivation 384-well platforms, demonstrated selective PDE inhibition and physiological activity (e.g. cAMP elevation) in secondary assays [24, 25, 26, 27, 28]. Similarly, the two (from 40,000 compounds) p38 α hits from the lethal expression assay both demonstrated activity in *in vitro* and in mammalian cell secondary assays [17], and two cyclic peptide hits identified as active against α -syn in yeast prevented dopaminergic neuron loss in a *Caenorhabditis elegans* Parkinson's model [19].

In assays designed to identify anti-infectives, secondary assays often involve establishing activity against the pathogen. For example, of the specific hits identified by Bilsland *et al* (2013) in the multiplexed fluorescent *S. cerevisiae* substitution platform, 50% proved cytotoxic to the protozoan parasite *Trypanosoma brucei in cellulo* [37]. However, this did not directly prove that the target was attenuated, in other work approaches involving *in vitro* and cell-based assays have demonstrated that the anti-parasitic hits generated from yeast-based screens do actually hit the intended target [21, 38, 41].

The necessity of the secondary assay approach, target directed and across both *in vitro* and *in cellulo* platforms, was illustrated very clearly in one of the very first yeast-based HTS campaigns from 1997 [35]. Klein *et al* identified a single, *selective*, compound following HTS and counter screening in conditions where the target, helminth ODC, is redundant. However, this hit failed to inhibit the target *in vitro* instead, perhaps, inhibiting a yeast enzyme involved in spermidine and spermine synthesis [35].

5. Conclusion:

As discussed above, yeast-based screening methodologies offer considerable benefits for HTS towards drug discovery (Table 1). Using their genetic tool box, yeast-based assays that facilitate a target-directed approach within a eukaryotic cell environment can be engineered to *bridge the gap between phenotypic and biochemical assays for HTS*. Such yeast-based

assays are robust (Z-factor ≥ 0.5 [13, 16, 26, 31, 41]) and cost effective - using simple “mix and measure” approaches with output measured by monitoring cell growth in liquid media. Although most of those reported achieve this via optical density, more recent yeast-based HTS has employed fluorometric responses [21, 36, 37, 38, 41]. This has enabled miniaturization and high content screening using 384-well [21, 36, 37, 38] and, recently 1536-well [41] platforms.

Although yeast-based screens have limitations (Table 1), the capacity for ultra-HTS of millions of compounds against a target in a eukaryotic cellular context, when allied with necessary counter and secondary screens, makes yeast a promising tool for the discovery of *drug-like* hit compounds.

Expert opinion:

The capacity to screen for specific target inhibition in a manipulable and well-controlled eukaryotic cellular context, meaning that yeast-based HTS combines many of the advantages of conventional *in vitro* biochemical and phenotypic screening approaches (Table 1). The use of a relatively simple eukaryotic *vehicle* also reduces many of the confounding effects caused by biological pathway redundancies in higher organisms. However, since the first reported yeast-based HTS in 1995 [12] less than 30 (many iterative) such campaigns have been published in the scientific literature (Table 2), a rate of little more than 1 per year. This relatively low level of visible activity is a limitation on development of these platforms, which of course have challenges such as the presence of a thick cell wall and highly expressed efflux pumps which reduce assay sensitivity. Although, genetic and chemical approaches to overcome these have been employed [16, 51] their use, and so validation, is limited. Furthermore, the use of sensitive fluorescent approaches and miniaturization towards ultra-HTS is recent and only deployed for a limited number of targets [21, 36, 37, 38, 41].

This lack of evidential research explains why no record of lead to hit drug discovery programmes stemming from yeast-based HTS could be identified in the literature. Although, importantly, Yumanity Therapeutics (co-founded by Susan Linquist [18, 19, 20]) have very recently (September 2018) issued a press release reporting the first clinical candidate (YTX-7739) for Parkinson’s disease discovered through their proprietary integrated discovery platform which includes a yeast-based component. Furthermore, very recent work has demonstrated the tractability of these HTS systems for screening *hard to purify/assay* proteins (e.g. transmembrane enzymes involved in lipid biosynthesis) in a robust miniaturized (1536-well) platform against a 1.8 million compound library [41]. This demonstrated that yeast-based platforms have huge potential to be deployed in industrial drug discovery, for the reasons cited above coupled with the fact that these rapidly growing, simple eukaryotes are cheap and easy

to cultivate and manipulate. To fully exploit the opportunities yeast-based HTS provides a number of challenges need to be addressed. Whilst 1536-well ultra-HTS has been reported [41], the fluorescent signal measured relied upon the addition of an expensive external agent (FDGlu) which required additional buffering [41]. The use of fluorescent proteins (either tagged target or expressed independently) has been reported and used to multiplex yeast-based HTS platforms [36, 37, 38]. This has great potential advantages, however the differential fluorescence seen in cell population expressing these proteins from the transformed plasmid [37] would prove problematic in any effort to achieve ultra-HTS. Indeed, in our ultra-HTS efforts [41] we abandoned this approach (Mina and Denny, unpublished). The use of clonal, stable yeast cell lines with integrated fluorescent markers should overcome this limitation and open the door for multiplexed ultra-HTS which would allow the selection of target specific compounds in a relatively cheap and efficient manner.

Therefore, whilst the future is difficult to predict, yeast-based platforms are well positioned to take their place in the armoury of drug discovery techniques in coming years, alongside conventional *in vitro* biochemical and phenotypic approaches, and fragment-based [52] and virtual screening [53, 54]. Whilst this may well happen out of sight of the academic community, such an increase in activity will increase the probability of compound hits entering hit to lead and preclinical trials programmes.

Article Highlights Box:

- Yeast-based screening has the potential to *bridge the gap between phenotypic and biochemical assays for HTS*
- Lethal expression, transactivation and substitution yeast-based assays have all been effectively employed in HTS, including ultra HTS
- Exploiting the genetic tractability of yeast to overcome platform limitations is possible, e.g. deletion of efflux pumps and use of fluorescent markers for multiplexing
- Recent developments, including the identification of a clinical candidate for Parkinson's disease, have shown that yeast-based HTS can take its place amongst the currently utilized drug discovery platforms

References:

* = of interest, ** = of considerable interest

1. An WF, Tolliday N. Cell-based assays for high-throughput screening. *Mol Biotechnol.* 2010 Jun;45(2):180-6. doi: 10.1007/s12033-010-9251-z. PubMed PMID: 20151227; eng.

- 2.* Denny PW, Steel PG. Yeast as a Potential Vehicle for Neglected Tropical Disease Drug Discovery. *J Biomol Screen*. 2014 Aug 13. doi: 1087057114546552 [pii] 10.1177/1087057114546552. PubMed PMID: 25121554; Eng.
 3. Norcliffe JL, Alvarez-Ruiz E, Martin-Plaza JJ, et al. The utility of yeast as a tool for cell-based, target-directed high-throughput screening. *Parasitology*. 2014 Jan;141(1):8-16. doi: S0031182013000425 [pii] 10.1017/S0031182013000425. PubMed PMID: 23611102; eng.
- Comprehensive review of the most widely utilised yeast-based platforms, including illustrations of the cell technologies employed.**
4. Simon JA, Bedalov A. Yeast as a model system for anticancer drug discovery. *Nat Rev Cancer*. 2004 Jun;4(6):481-92. doi: 10.1038/nrc1372 nrc1372 [pii]. PubMed PMID: 15170450; eng.
 5. Limenitakis J, Soldati-Favre D. Functional genetics in Apicomplexa: potentials and limits. *FEBS Lett*. 2011 Jun 6;585(11):1579-88. doi: S0014-5793(11)00340-1 [pii] 10.1016/j.febslet.2011.05.002. PubMed PMID: 21557944; eng.
 6. Barberis A, Gunde T, Berset C, et al. Yeast as a screening tool. *Drug Disc Today*. 2005;2(2):187-192.
 7. Castrillo JI, Oliver SG. Yeast systems biology: the challenge of eukaryotic complexity. *Methods Mol Biol*. 2011;759:3-28. doi: 10.1007/978-1-61779-173-4_1. PubMed PMID: 21863478; eng.
 8. Hughes TR. Yeast and drug discovery. *Funct Integr Genomics*. 2002 Sep;2(4-5):199-211. doi: 10.1007/s10142-002-0059-1. PubMed PMID: 12192593; eng.
 9. Munder T, Hinnen A. Yeast cells as tools for target-oriented screening. *Appl Microbiol Biotechnol*. 1999 Sep;52(3):311-20. PubMed PMID: 10531642; eng.
 10. Hamdi A, Colas P. Yeast two-hybrid methods and their applications in drug discovery. *Trends Pharmacol Sci*. 2012 Feb;33(2):109-18. doi: 10.1016/j.tips.2011.10.008. PubMed PMID: 22130009.
 11. Lin Y, Li Y, Zhu Y, et al. Identification of antituberculosis agents that target ribosomal protein interactions using a yeast two-hybrid system. *Proc Natl Acad Sci U S A*. 2012 Oct 23;109(43):17412-7. doi: 10.1073/pnas.1110271109. PubMed PMID: 23045703; PubMed Central PMCID: PMC3491523.
 - 12.* Kurtz S, Luo G, Hahnenberger KM, et al. Growth impairment resulting from expression of influenza virus M2 protein in *Saccharomyces cerevisiae*: identification of a novel inhibitor of influenza virus. *Antimicrob Agents Chemother*. 1995 Oct;39(10):2204-9. PubMed PMID: 8619568; PubMed Central PMCID: PMC162915. eng.

First literature report of the use a yeast-based screen for HTS.

13. Benko Z, Elder RT, Liang D, et al. Fission yeast as a HTS platform for molecular probes of HIV-1 Vpr-induced cell death. *Int J HTS*. 2010;1:151-162.
14. Lee JC, Kumar S, Griswold DE, et al. Inhibition of p38 MAP kinase as a therapeutic strategy. *Immunopharmacology*. 2000 May;47(2-3):185-201. PubMed PMID: 10878289.
15. Wu G. Recent progress in phosphoinositide 3-kinases: oncogenic properties and prognostic and therapeutic implications. *Curr Protein Pept Sci*. 2010 Sep;11(6):425-35. doi: CPPS-57 [pii]. PubMed PMID: 20491626; eng.
16. Fernandez-Acero T, Rodriguez-Escudero I, Vicente F, et al. A yeast-based in vivo bioassay to screen for class I phosphatidylinositol 3-kinase specific inhibitors. *J Biomol Screen*. 2012 Sep;17(8):1018-29. doi: 1087057112450051 [pii] 10.1177/1087057112450051. PubMed PMID: 22706348; eng.
17. Friedmann Y, Shriki A, Bennett ER, et al. JX401, A p38alpha inhibitor containing a 4-benzylpiperidine motif, identified via a novel screening system in yeast. *Mol Pharmacol*. 2006 Oct;70(4):1395-405. doi: mol.106.022962 [pii] 10.1124/mol.106.022962. PubMed PMID: 16847144; eng.
18. Griffioen G, Duhamel H, Van Damme N, et al. A yeast-based model of alpha-synucleinopathy identifies compounds with therapeutic potential. *Biochim Biophys Acta*. 2006 Mar;1762(3):312-8. doi: 10.1016/j.bbadis.2005.11.009. PubMed PMID: 16413174.
- 19.** Kritzer JA, Hamamichi S, McCaffery JM, et al. Rapid selection of cyclic peptides that reduce alpha-synuclein toxicity in yeast and animal models. *Nat Chem Biol*. 2009 Sep;5(9):655-63. doi: 10.1038/nchembio.193. PubMed PMID: 19597508; PubMed Central PMCID: PMC2729362.

Integration of SICLOPPS technology into yeast-based HTS platform to facilitate the screening of 5 million cyclic peptides.

20. Su LJ, Auluck PK, Outeiro TF, et al. Compounds from an unbiased chemical screen reverse both ER-to-Golgi trafficking defects and mitochondrial dysfunction in Parkinson's disease models. *Dis Model Mech*. 2010 Mar-Apr;3(3-4):194-208. doi: 10.1242/dmm.004267. PubMed PMID: 20038714; PubMed Central PMCID: PMC2869493.
21. Frame IJ, Deniskin R, Rinderspacher A, et al. Yeast-based high-throughput screen identifies Plasmodium falciparum equilibrative nucleoside transporter 1 inhibitors that kill malaria parasites. *ACS Chem Biol*. 2015 Mar 20;10(3):775-83. doi: 10.1021/cb500981y. PubMed PMID: 25602169; PubMed Central PMCID: PMC4369170.

22. Grozinger CM, Chao ED, Blackwell HE, et al. Identification of a class of small molecule inhibitors of the sirtuin family of NAD-dependent deacetylases by phenotypic screening. *J Biol Chem.* 2001 Oct 19;276(42):38837-43. doi: 10.1074/jbc.M106779200. PubMed PMID: 11483616.
23. Middendorp O, Ortler C, Neumann U, et al. Yeast growth selection system for the identification of cell-active inhibitors of beta-secretase. *Biochim Biophys Acta.* 2004 Sep 6;1674(1):29-39. doi: 10.1016/j.bbagen.2004.05.004 S0304416504001175 [pii]. PubMed PMID: 15342111; eng.
24. Alaamery MA, Wyman AR, Ivey FD, et al. New classes of PDE7 inhibitors identified by a fission yeast-based HTS. *J Biomol Screen.* 2010 Apr;15(4):359-67. doi: 10.1177/1087057110362100. PubMed PMID: 20228279; PubMed Central PMCID: PMC2854023.
25. Ceyhan O, Birsoy K, Hoffman CS. Identification of biologically active PDE11-selective inhibitors using a yeast-based high-throughput screen. *Chem Biol.* 2012 Jan 27;19(1):155-63. doi: S1074-5521(11)00462-5 [pii] 10.1016/j.chembiol.2011.12.010. PubMed PMID: 22284362; eng.
26. de Medeiros AS, Wyman AR, Alaamery MA, et al. Identification and characterization of a potent and biologically-active PDE4/7 inhibitor via fission yeast-based assays. *Cell Signal.* 2017 Dec;40:73-80. doi: 10.1016/j.cellsig.2017.08.011. PubMed PMID: 28867658; PubMed Central PMCID: PMC5651194.
27. Demirbas D, Wyman AR, Shimizu-Albergine M, et al. A yeast-based chemical screen identifies a PDE inhibitor that elevates steroidogenesis in mouse Leydig cells via PDE8 and PDE4 inhibition. *PLoS One.* 2013;8(8):e71279. doi: 10.1371/journal.pone.0071279 PONE-D-13-17771 [pii]. PubMed PMID: 23967182; PubMed Central PMCID: PMC3743849. eng.
28. Ivey FD, Wang L, Demirbas D, et al. Development of a fission yeast-based high-throughput screen to identify chemical regulators of cAMP phosphodiesterases. *J Biomol Screen.* 2008 Jan;13(1):62-71. doi: 1087057107312127 [pii] 10.1177/1087057107312127. PubMed PMID: 18227226; PubMed Central PMCID: PMC2851203. eng.
29. Bender AT, Beavo JA. Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use. *Pharmacol Rev.* 2006 Sep;58(3):488-520. doi: 58/3/488 [pii] 10.1124/pr.58.3.5. PubMed PMID: 16968949; eng.
30. Conti M, Beavo J. Biochemistry and physiology of cyclic nucleotide phosphodiesterases: essential components in cyclic nucleotide signaling. *Annu Rev Biochem.* 2007;76:481-511. doi: 10.1146/annurev.biochem.76.060305.150444. PubMed PMID: 17376027; eng.

31. Demirbas D, Ceyhan O, Wyman AR, et al. A fission yeast-based platform for phosphodiesterase inhibitor HTSs and analyses of phosphodiesterase activity. *Handb Exp Pharmacol*. 2011 (204):135-49. doi: 10.1007/978-3-642-17969-3_5. PubMed PMID: 21695638; eng.
32. Zaks-Makhina E, Kim Y, Aizenman E, et al. Novel neuroprotective K⁺ channel inhibitor identified by high-throughput screening in yeast. *Mol Pharmacol*. 2004 Jan;65(1):214-9. doi: 10.1124/mol.65.1.214
65/1/214 [pii]. PubMed PMID: 14722253; PubMed Central PMCID: PMC2950965. eng.
33. Marjanovic J, Chalupska D, Patenode C, et al. Recombinant yeast screen for new inhibitors of human acetyl-CoA carboxylase 2 identifies potential drugs to treat obesity. *Proc Natl Acad Sci U S A*. 2010 May 18;107(20):9093-8. doi: 1003721107 [pii] 10.1073/pnas.1003721107. PubMed PMID: 20439761; PubMed Central PMCID: PMC2889071. eng.
34. Zhang Y, Tian D, Matsuyama H, et al. Human Adenine Nucleotide Translocase (ANT) Modulators Identified by High-Throughput Screening of Transgenic Yeast. *J Biomol Screen*. 2016 Apr;21(4):381-90. doi: 10.1177/1087057115624637. PubMed PMID: 26746582.
35. Klein RD, Favreau MA, Alexander-Bowman SJ, et al. *Haemonchus contortus*: cloning and functional expression of a cDNA encoding ornithine decarboxylase and development of a screen for inhibitors. *Exp Parasitol*. 1997 Nov;87(3):171-84. doi: S0014-4894(97)94213-2 [pii] 10.1006/expr.1997.4213. PubMed PMID: 9371082; eng.
36. Bilsland E, Bean DM, Devaney E, et al. Yeast-Based High-Throughput Screens to Identify Novel Compounds Active against *Brugia malayi*. *PLoS Negl Trop Dis*. 2016 Jan;10(1):e0004401. doi: 10.1371/journal.pntd.0004401. PubMed PMID: 26812604; PubMed Central PMCID: PMCPMC4727890.
- 37.** Bilsland E, Sparkes A, Williams K, et al. Yeast-based automated high-throughput screens to identify anti-parasitic lead compounds. *Open Biol*. 2013 Feb;3(2):120158. doi: rsob.120158 [pii] 10.1098/rsob.120158. PubMed PMID: 23446112; PubMed Central PMCID: PMC3603448. eng.

Use of fluorescent portein tags to facilitate multiplexing for a yeast-based assay in 384-well plates.

38. Bilsland E, van Vliet L, Williams K, et al. Plasmodium dihydrofolate reductase is a second enzyme target for the antimalarial action of triclosan. *Sci Rep*. 2018 Jan 18;8(1):1038. doi: 10.1038/s41598-018-19549-x. PubMed PMID: 29348637; PubMed Central PMCID: PMCPMC5773535.

39. Denny PW, Shams-Eldin H, Price HP, et al. The protozoan inositol phosphorylceramide synthase: A novel drug target which defines a new class of sphingolipid synthase J Biol Chem. 2006;281:28200-28209.
40. Mina JG, Pan SY, Wansadhipathi NK, et al. The Trypanosoma brucei sphingolipid synthase, an essential enzyme and drug target. Mol Biochem Parasitol. 2009 Nov;168(1):16-23. doi: S0166-6851(09)00161-3 [pii]10.1016/j.molbiopara.2009.06.002. PubMed PMID: 19545591; eng.
- 41.** Norcliffe JL, Mina JG, Alvarez E, et al. Identifying inhibitors of the Leishmania inositol phosphorylceramide synthase with antiprotozoal activity using a yeast-based assay and ultra-high throughput screening platform. Sci Rep. 2018 Mar 2;8(1):3938. doi: 10.1038/s41598-018-22063-9. PubMed PMID: 29500420; PubMed Central PMCID: PMC5834442.
First published use of a yeast-based platform in ultra HTS using 1536-well plates.
42. Mina JG, Thye JK, Alqaisi AQL, et al. Functional and phylogenetic evidence of a bacterial origin for the first enzyme in sphingolipid biosynthesis in a phylum of eukaryotic protozoan parasites. J Biol Chem. 2017 Jul 21;292(29):12208-12219. doi: 10.1074/jbc.M117.792374. PubMed PMID: 28578314; PubMed Central PMCID: PMC5519370.
43. Mina JGM, Denny PW. Everybody needs sphingolipids, right! Mining for new drug targets in protozoan sphingolipid biosynthesis. Parasitology. 2018 Feb;145(2):134-147. doi: 10.1017/S0031182017001081. PubMed PMID: 28637533; PubMed Central PMCID: PMC5964470.
44. Pratt S, Wansadhipathi-Kannangara NK, Bruce CR, et al. Sphingolipid synthesis and scavenging in the intracellular apicomplexan parasite, Toxoplasma gondii. Mol Biochem Parasitol. 2013 Jan;187(1):43-51. doi: 10.1016/j.molbiopara.2012.11.007. PubMed PMID: 23246819; PubMed Central PMCID: PMC3629565.
45. Young SA, Mina JG, Denny PW, et al. Sphingolipid and ceramide homeostasis: potential therapeutic targets. Biochem Res Int. 2012;2012:248135. doi: 10.1155/2012/248135. PubMed PMID: 22400113; PubMed Central PMCID: PMC3286894.
46. Bach S, Talarek N, Andrieu T, et al. Isolation of drugs active against mammalian prions using a yeast-based screening assay. Nat Biotechnol. 2003 Sep;21(9):1075-81. doi: 10.1038/nbt855. PubMed PMID: 12910243.
47. Bach S, Tribouillard D, Talarek N, et al. A yeast-based assay to isolate drugs active against mammalian prions. Methods. 2006 May;39(1):72-7. doi: 10.1016/j.ymeth.2006.04.005. PubMed PMID: 16750390.

48. Couplan E, Aiyar RS, Kucharczyk R, et al. A yeast-based assay identifies drugs active against human mitochondrial disorders. *Proc Natl Acad Sci U S A*. 2011 Jul 19;108(29):11989-94. doi: 10.1073/pnas.1101478108. PubMed PMID: 21715656; PubMed Central PMCID: PMC3141935.
49. Young K, Lin S, Sun L, et al. Identification of a calcium channel modulator using a high throughput yeast two-hybrid screen. *Nat Biotechnol*. 1998 Oct;16(10):946-50. doi: 10.1038/nbt1098-946. PubMed PMID: 9788351; eng.
50. Stagoj MN, Komel R, Comino A. Microtiter plate assay of yeast cell number using the fluorescent dye calcofluor white M2R. *Biotechniques*. 2004 Mar;36(3):380-2. doi: 10.2144/04363BM01. PubMed PMID: 15038150.
51. Bilsland E, Pir P, Gutteridge A, et al. Functional expression of parasite drug targets and their human orthologs in yeast. *PLoS Negl Trop Dis*. 2011 Oct;5(10):e1320. doi: 10.1371/journal.pntd.0001320 PNTD-D-11-00499 [pii]. PubMed PMID: 21991399; PubMed Central PMCID: PMC3186757. eng.
52. Marchetti C, Chan DSH, Coyne AG, et al. Fragment-based approaches to TB drugs. *Parasitology*. 2018 Feb;145(2):184-195. doi: 10.1017/S0031182016001876. PubMed PMID: 27804891.
53. Cardew EM, Verlinde C, Pohl E. The calcium-dependent protein kinase 1 from *Toxoplasma gondii* as target for structure-based drug design. *Parasitology*. 2018 Feb;145(2):210-218. doi: 10.1017/S0031182017001901. PubMed PMID: 29191253; PubMed Central PMCID: PMC5964469.
54. Tatum NJ, Liebeschuetz JW, Cole JC, et al. New active leads for tuberculosis booster drugs by structure-based drug discovery. *Org Biomol Chem*. 2017 Dec 13;15(48):10245-10255. doi: 10.1039/c7ob00910k. PubMed PMID: 29182187.

Figures and tables:

Table 1

	Advantages	Disadvantages
<i>In vitro</i> HTS	<p>Possibility of simple, specific and sensitive assay system</p> <p>Facilitates SAR based on molecular recognition of the target</p>	<p>Requires purified target, limiting assay of hard to purify/assay targets such as transmembrane enzymes</p> <p>Expensive due to necessity for protein purification etc</p> <p>Requirement for suitable substrates</p>
Phenotypic HTS	<p>Does not require purified target</p> <p>Provides specific cellular context allowing early selection of drug-like compounds</p> <p>Naïve approach challenging all targets at once in an unbiased manner</p>	<p>Relatively insensitive due to drug pumps and membrane barriers</p> <p>Expensive or inability to culture relevant cell type</p> <p>Reliance on non-target specific, phenotypic output</p> <p>SAR is not assisted by target knowledge. Target deconvolution is approached <i>a posteriori</i></p>
Yeast-based HTS	<p>Does not require purified target</p> <p>Provides axenic eukaryotic cellular context allowing early selection of drug-like compounds</p> <p>Ease of manipulation and speed of growth</p> <p>Straightforward genetic manipulation for generic assay platforms</p> <p>Ease of discrimination of false positive hits</p> <p>Low cost of culture</p>	<p>Relatively insensitive due to drug pumps, membrane barriers and the thick cell wall</p> <p>Often reliant on the ability of an heterologous protein to be functional in an axenic system</p> <p>Target protein is tested in a non-native cellular <i>milieu</i></p>

Table 2

Organism	Disease/effect	Target(s)	Assay	Library/hits	Outcome	Reference
Influenza virus	Influenza	Ion-channel forming M ₂	Lethal expression in <i>Saccharomyces cerevisiae</i> , 96-well plates	'Compounds drawn from the Bristol-Myers Squibb compound collection', 1 hit	<i>In vivo</i> activity in plaque assay (μM)	Kurtz <i>et al</i> , 1995 [12]
<i>Haemonchus contortus</i>	Anaemia and oedema in ruminants	Orthithine decarboxylase	Substitution in <i>S. cerevisiae</i> , 96-well plates	'A subset of a collection of synthetic compounds', (90,000), 1 hit	Single hit shown to be not on target <i>in vitro</i> and <i>in cellulo</i>	Klein <i>et al</i> , 1997 [35]
Human	Neurological trauma	Calcium channel (subunits α1B and β3)	Transactivation (2 hybrid) in <i>S. cerevisiae</i> , agar plates	Subset American Home Products Corporate (156,000), 10 hits	Specific <i>in cellulo</i> activity (μM)	Young <i>et al</i> , 1998 [49]
Human	Cancer, diabetes, cardiovascular diseases, neuro-degenerative diseases	Sirtuin (SIRT2)	Transactivation in <i>S. cerevisiae</i> , 96- and 384-well plates	IICB Diversity and Chem-Bridge (1,600), 3 hits	Specific <i>in vitro</i> , <i>in cellulo</i> (mammalian) and <i>in vivo</i> (plant) activity (μM)	Grozingier <i>et al</i> , 2001 ⁴ [22]
Human	Prion diseases	Prion protein (PrP)	Lethal expression in <i>S. cerevisiae</i> , agar plates	'Synthetic and natural products purified from various sources by academic laboratories' (2,500), 6 hits	Specific <i>in cellulo</i> activity in yeast and mammalian systems (μM)	Bach <i>et al</i> , 2003 [46], 2006 [47]
Human	Alzheimer's disease	β-secretase	Transactivation in <i>S. cerevisiae</i> , 96-well plates	'Chemical compound library' (15,000), 13 hits	Specific <i>in cellulo</i> activity (μM)	Middendorp <i>et al</i> , 2004 [23]
Human	Inflammatory diseases, cancer, congestive heart disease	protein kinase p38α	Lethal expression in <i>S. cerevisiae</i> , 96-well plates	ChemDiv combinatorial (40,000), 2 hits	On target <i>in vitro</i> and <i>in cellulo</i> activity (nM-μM)	Freidmann <i>et al</i> , 2006 [17]
Human	Parkinson's disease	α-synuclein (α-syn)	Lethal expression in <i>S. cerevisiae</i> , agar, 96- and 384-well plates	'collection of 10,022 diverse compounds' from ComGenex and Sigma Aldrich Expressed cyclic peptide library (5M) 'Over 115,000 compounds from various collections, including commercial libraries, natural products and National Cancer Institute collections'	Active flavonoids identified Specific <i>in cellulo</i> activity in nematode model system (μM) Specific <i>in cellulo</i> activity in nematode and mammalian model systems (μM)	Griffioen <i>et al</i> , 2006 [18] Kritzer <i>et al</i> , 2009 [19] Su <i>et al</i> , 2010 [20]

Human	Neurological dis- eases, inflammatory diseases, metabolic diseases, memory loss, cancer, erectile dysfunction	Phosphodiesterase (PDE) 2A/4A/4B/8	Transactivation in <i>S. pombe</i> , 384-well plates	'Subset' Prestwick Bioactive & Microsource Spectrum (3120), 151 hits	On target (PDE4B) <i>in cellulo</i> activity (μM)	Ivey <i>et al</i> , 2008 [28]
		PDE7		'Commercial chemical libraries', (48,176), 5 hits	On target <i>in cellulo</i> and <i>in vitro</i> activity (μM)	Alaamery <i>et al</i> , 2010 [24]
		PDE11		Including Actinol TimTec1, ChemBridge1,3, ChemDiv3&6 (198,382), 39 hits	On target <i>in cellulo</i> and <i>in vitro</i> activity (μM)	Cayhan <i>et al</i> , 2011[25]
		PDE4/8		BIOMOL2 ICCB, Prestwick1, NINDS2, Asinex1, Biomol-TimTec1, Bionet1-2, CEREP, ChemBridge3, ChemDiv 1-4&6, Enamine1-2, I.F. Lab2, Life Chemicals1, MayBridge1-5, MixCommercial1-3&5, and Peakdale (222,711), 367 hits	On target <i>in vitro</i> activity (nM) and elevation of cell testosterone and progesterone <i>in cellulo</i> (μM)	Demirbas <i>et al</i> , 2013 [27]
		PDE 4/7		Broad Institute (undefined), 163-1610 hits	On target <i>in vitro</i> activity (nM), anti-inflammatory action <i>in cellulo</i> (μM) and molecular docking (low energy)	Santos de Medeiros <i>et al</i> , 2017 [26]
Human	Anderson syndrome	Potassium channel (Kir2.1)	Substitution in <i>S. cerevisiae</i> , 96-well plates	MicroSource Natural Products Library (720), 1 hit	On target <i>in cellulo</i> activity in yeast and mammalian cells (μM)	Zaks-Makhina <i>et al</i> , 2009 [32]
Human	Obesity, cancer	Acetyl-CoA carboxylase 1/2 (ACC1/2)	Substitution in <i>S. cerevisiae</i> , 96-well plates	ChemDiv: 'ACC1 Structure-Activity Relationship Library' (3,068); 'Diverse screening library' (30,000); 'ACC2 Analog Screening Library' (828), 38 hits	On target <i>in vitro</i> activity selective for AAC2 (μM)	Marjanovic <i>et al</i> , 2010 [33]
HIV-1	AIDS	Viral protein R	Lethal expression in <i>Saccharomyces pombe</i> , 384-well and agar plates	Microsource Spectrum Collection (2,000), 1 hit	Confirmation of anti-apoptotic effect (μM)	Benko <i>et al</i> , 2010 [13]
Human	Mitochondrial disease	Mitochondrial ATP synthase (ATP6)	Lethal expression in <i>S. cerevisiae</i> , agar plates	'Compounds from various chemical libraries' (12,000), 10 hits	On target <i>in cellulo</i> activity in mammalian cells (μM)	Couplan <i>et al</i> , 2011 [48]
Human	Cancer, autoimmune diseases, metabolic disorders, atherosclerosis cardiovascular disease	Phosphatidyl-inositol 3-kinase (PI3K)	Lethal expression in <i>Saccharomyces cerevisiae</i> , 96-well plates	'Collection of natural products of microbial origin' (9,600), 55 hits	On target <i>in cellulo</i> (μM)	Fernandez-Acero <i>et al</i> , 2012 [16]

<i>Plasmodium</i> spp, <i>Schistosoma masoni</i> , <i>Trypanosoma</i> spp, <i>Leishmania major</i>	Malaria Schistosomiasis African/American trypanosomiasis Leishmaniasis	Dihydrofolate reductase (DHFR), N-myristoyl-transferase (NMT), Phosphoglycerate kinase (PGK)	Substitution in <i>S. cerevisiae</i> , multiplexed, 384-well plates	Maybridge Hitfinder (14,400), 445 hits	Anti- <i>T. brucei</i> activity <i>in cellulo</i> (nM)	Bilsland <i>et al</i> , 2013 [37]
Human	Cancer, diabetes, cardiovascular disease, obesity, male contraception	Adenine Nucleotide Translocase 4 (ANT4)	Substitution in <i>S. cerevisiae</i> , 384-well plates	65,000 compounds from 'commercial sources', 19 hits	On target activity <i>in vitro</i> (µM)	Zhang <i>et al</i> , 2016 [34]
<i>P. falciparum</i>	Malaria	Nucleoside Transporter 1	Substitution in <i>S. cerevisiae</i> , 384-well plates	'several chemical libraries' (64,560), 171 hits	On target cellular activity (nM) and anti-parasitic activity against infected erythrocytes (µM)	Frame <i>et al</i> , 2015 [21]
<i>Brugia malayi</i>	Lymphatic filariasis	NMT, PGK, triose-phosphate isomerase, adenosyl homocysteinase, thymidylate synthetase, ribose-5-phosphate isomerase, deoxyhypusine synthase, CDP-alcohol phosphatidyl transferase	Substitution in <i>S. cerevisiae</i> , multiplexed, 384-well plates	Malaria Box (400), 9 hits	Anti- <i>B. malayi</i> activity <i>in cellulo</i> (µM)	Bilsland <i>et al</i> , 2016 [36]
<i>Leishmania</i> spp	Leishmaniasis	Inositol phosphoryl-ceramide (IPC) synthase	Substitution in <i>S. cerevisiae</i> , 1536-well plates	GSK library (1.8M), 500 hits	On target <i>in vitro</i> and <i>in cellulo</i> activity (nM-µM) and anti-leishmanial activity <i>in cellulo</i> (nM)	Norcliffe <i>et al</i> , 2018 [41]
<i>Plasmodium</i> spp	Malaria	DHFR	Substitution in <i>S. cerevisiae</i> , multiplexed, 384-well plates.	Johns Hopkins FDA & foreign approved library (1,600), 1 hit	On target <i>in vitro</i> (nM), anti- <i>P. falciparum</i> activity <i>in cellulo</i> (µM) and molecular docking (low energy)	Bilsland <i>et al</i> , 2018 [38]